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(54) Title of the Invention:

CONTRAST MEDIA MADE FROM POLYALDEHYDES

(57) Abstract:

Microparticles consisting of biodegradable polymers, characterized in that they are made from polymerizable aldehydes, possibly containing copolymerizable additives and/or crosslinking agents, possibly surfactants or surfactant mixtures, gases and/or readily volatile liquids in free or bound form, coupling agents, possibly biomolecules or macromolecules bound via these coupling agents, and possibly diagnostically or therapeutically effective ingredients, that are suitable for use as ultrasonic contrast media.

CONTRAST MEDIA MADE FROM POLYALDEHYDES

The invention concerns the subject specified in the patent claims, namely, novel microparticles, pharmaceutical agents that contain these microparticles, their use in ultrasonic diagnosis, and methods for producing these microparticles and pharmaceutical agents.

It is known that contrast echocardiography is typically done by peripheral injection of solutions that contain fine gas bubbles [Roelandt, J., *Ultrasound Med. Biol.*, Vol. 8, pp. 471-492, 1982]. These gas bubbles are produced in physiologically suitable solutions, e.g., by shaking, by some other means of agitation, or by the addition of carbon dioxide. However, they are not uniform in number and size and their reproducibility is not satisfactory. What is more, they are generally not stabilized, and so do not last very long. Their average diameter is generally greater than that of erythrocytes, so they cannot pass through the pulmonary capillaries and then be used as contrast media for organs such as the left heart, liver, kidneys, or spleen. Furthermore, they are not suitable for quantitative measurements, because the ultrasonic echo they produce arises from several processes that cannot be separated

from one another, such as bubble formation, coalescence, and dissolution. It is therefore not possible, for example, to use these ultrasonic contrast media to obtain information about transit times by measuring the course of the contrast medium in the myocardium.

European Patent No. 131,540 describes the stabilization of gas bubbles with sugar. Although this does improve the reproducibility and homogeneity of the contrast effect, these bubbles do not survive passage through the lungs.

European Patents No. 122,624 and No. 123,235 describe the addition of surfactants to improve the effect of sugars, sugar alcohols, and salts in stabilizing gas bubbles. These ultrasonic contrast media permit passage through the pulmonary capillaries and allow the possibility of visualizing the arterial branches and various organs such as the liver or spleen. However, the contrast effect here is limited to the vascular lumen, because the bubbles are not taken up by the tissue cells.

None of the ultrasonic contrast media known to date remain intact in the body for long. Therefore, visualization of organs with adequate signal intensity by selective accumulation following intravenous administration is not possible, nor are quantitative measurements.

An encapsulation of gases, such as air, as an ultrasonic contrast medium is described in West German Offenlegungsschrift No. 3,803,972. The wall material used in this process consists of biodegradable synthetic material, cyanoacrylate and polylactide being especially good examples.

These microparticles, however, are difficult to produce, especially on a relatively large scale and in terms of their workup. In particular, their broad particle-size distributions are a disadvantage.

The objective was therefore to create ultrasonic contrast media that do

not suffer from these disadvantages. This objective is achieved by the present invention, that is, by the provision of microparticles according to the invention.

These microparticles consist of biodegradable polymers, characterized in that they are made from polymerizable aldehydes, possibly containing copolymerizable additives and/or crosslinking agents, possibly surfactants or surfactant mixtures, gases and/or readily volatile liquids in free or linked form, coupling agents, possibly biomolecules or macromolecules linked via these coupling agents, and possibly diagnostically or therapeutically effective ingredients.

All the polymers (homopolyaldehydes or copolyaldehydes) according to the invention generally have molecular masses of 1,000-30,000 daltons, and preferably 1,000-12,000 daltons. The particles are degradable in both the blood and plasma.

The main polymerized aldehyde constituents of the microparticles are selected from the following polymerizable aldehydes:

I. α,β-unsaturated aldehydes, for example:

acrolein;

crotonaldehyde;

propionaldehyde;

II. α-substituted acrolein derivatives, for example:

α-methylacrolein;

α-chloroacrolein;

α-phenylacrolein;

α-ethylacrolein;

α-isopropylacrolein;

α-*N*-butylacrolein;

α-*N*-propylacrolein;

III. Dialdehydes, for example:

glutaraldehyde, succinaldehyde or their derivatives or mixtures of these with copolymerizable additives, for example:

α-substituted acroleins;

β-substituted acroleins;

ethylcyanoacrylates;

methylcyanoacrylates;

butylcyanoacrylates;

hexylcyanoacrylates;

methyl methacrylates;

vinyl alcohols;

acrylic acids;

methacrylic acids;

acrylic acid chlorides;

methacrylic acid chlorides;

acrylonitrile;

methacrylonitriles;

acrylamides;

substituted acrylamides;

hydroxymethyl methacrylates;

mesityl oxide;

2-dimethylaminoethyl methacrylates;

vinylpyridines; and

N-vinyl-2-pyrrolidinone.

Acrolein and glutaraldehyde are the constituents of first choice.

Suitable surfactants that can be used as constituents of the microparticles can be selected from ionogenic or non-ionogenic surface-active substances (surfactants), for example:

polyethylene oxide;

polyoxyethylene polyoxypropylenes such as Pluronic® F 68, Pluronic® F 108, Pluronic® F 127;

polyethylene glycol, Poloxamin 908, Polaxamer 407;

carboxylic acid salts, for example, sodium oleate;

polyoxyethylene fatty acid esters, for example:

polyoxyethylene stearate;

sodium dioctyl sulfosuccinate;

polyglutaraldehyde sodium hydrogen sulfite adducts;

polyacrolein sodium hydrogen sulfite adducts; and

polyvinylsulfonic acid.

They may be used alone or as their mixtures.

The most preferred of the surfactants are:

polyglutaraldehyde sodium sulfite adducts; and the

polyacrolein sodium hydrogen sulfite adducts, Pluronic® F 68, Pluronic® F 108, and Pluronic® F 127.

If the polymerizable aldehydes used to make up the microparticles have surface-active properties, the use of surfactants can be omitted. Glutaraldehyde may be mentioned as an example of a surface-active aldehyde.

Suitable substances for use in the present invention as the gases or readily volatile liquids, preferably liquids with a boiling point below 60°C, to be contained in the microparticles in free or bound form include:

ammonia;

air;

noble gases (helium, neon, argon, xenon, and krypton);

sulfur halides, for example, sulfur hexafluoride;

nitrogen;

carbon oxides;

oxygen;

hydrogen;

hydrocarbons or their mixtures, for example:

methane;

ethane;

propane;

butane;

pentane;

neopentane;

isopentane;

cyclopentane;

ethylene;

propylene;

acetylene;

3,3-dimethyl-1-butyne;

2,3-pentadiene;

2-methyl-2-butene;

2-methyl-1,3-butadiene;

2-butyne;

2-methyl-1-butene;

3-methyl-1-butene;

halogenated hydrocarbons or mixtures, for example:

methylene chloride;

1,1-dichloroethylene;

isopropyl chloride;

dibromodifluoromethane;

bromomethane;

ethers, for example: dimethyl ether, diethyl ether, or fluorinated ether;

or compounds such as:

dimethylaminoacetone;

propylene oxide;

N-ethylmethylamine;

N-ethyldimethylamine; and

furan.

The constituents of choice are air, argon, xenon, sulfur hexafluoride, propane, butane, and furan.

Especially suitable coupling agents for use as constituents of the microparticles include:

I. Compounds that contain amino groups, for example;

hydroxylamine;

butylamine;

allylamine;

ethanolamine;

trishydroxymethylaminomethane;

3-amino-1-propanesulfonic acid;

5-aminopentanoic acid;

8-aminooctanoic acid;

D-glucosamine hydrochloride;

aminogalactose;

aminosorbitol;

aminomannitol;

diethylaminoethylamine;

anilines;

sulfonyl acid amide;

choline;

N-methylglucamine;

piperazine;

1,6-hexanediamine;

urea;

hydrazine;

glycine;

alanine;

lysine;

serine;

valine;

leucine;

peptides;

proteins;

albumin;

human serum albumin;

polylysine;

gelatins;

polyglycol amines;

amino polyols;

dextran sulfates with amino groups;

N-aminopolyethylene glycol (HO—PEG—NH_2);

N,*N'*-diaminopolyethylene glycol (NH_2—PEG—NH_2);

antibodies; and

immunoglobulins.

II. Compounds that contain acid groups, for example:

carboxylic acids;

acetic acid;

propionic acid;

butyric acid;

valeric acid;

caproic acid;

caprylic acid;

capric acid;

lauric acid;

myristic acid;

palmitic acid;

stearic acid;

oleic acid;

linoleic acid;

linolenic acid;

cyclohexane carboxylic acid;

phenylacetic acid;

benzoylacetic acid;

chlorobenzoic acid;

bromobenzoic acid;

nitrobenzoic acid;

ortho-phthalic acid;

meta-phthalic acid;

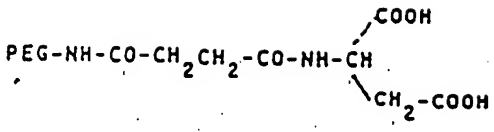
para-phthalic acid;

salicylic acid;

hydroxybenzoic acid;

aminobenzoic acid;

methoxybenzoic acid;



(PEG—*L*-asparagic acid);

PEG—*L*-glutamic acid;

PEG—*L*-DTPA;

PEG—*L*-EDTA;

III. Compounds that contain hydroxyl groups, for example:

alcohols;

methanol;

ethanol;

propanol;

butanol;

pentanol;

hexanol;

heptanol;

octanol;

decanol;

dodecanol;

tetradecanol;

hexadecanol;

octadecanol;

isopropyl alcohol;

isobutyl alcohol;

isopentyl aclohol;

cyclopentanol;

cyclohexanol;

crotyl alcohol;

benzyl alcohol;

phenyl alcohol;

diphenylmethanol;

triphenylmethanol;

cinnamic alcohol;

ethylene glycol;

1,3-propanediol;

glycerol;

pentaerythritol;

IV. Polymerizable substances, such as α,β-unsaturated aldehydes, for example:

acrolein;

crotonaldehyde;

propynaldehyde;

α-substituted acrolein derivatives, for example:

α-methylacrolein;

α-chloroacrolein;

α-phenylacrolein;

α-ethylacrolein;

α-isopropylacrolein;

α-butylacrolein;

α-propylacrolein;

dialdehydes, for example:

glutaraldehyde, succinaldehyde, or their derivatives, or mixtures of these with copolymerizable additives, for example:

α-substituted acroleins;

β-substituted acroleins;

ethylcyanoacrylates;

methylcyanoacrylates;

butyl acrylates;

hexylcyanoacrylates;

methyl methacrylates;

vinyl alcohols;

acrylic acids;

methacrylic acids;

acrylic acid chlorides;

acrylonitrile;

methacrylonitriles;

acrylamides;

substituted acrylamides;

hydroxymethyl methacrylates;

mesityl oxide;

2-dimethylaminoethyl methacrylates;

vinylpyridines; and

N-vinyl-2-pyrrolidinone.

Preferred among these are hydroxylamine, tris(hydroxymethyl)aminomethane, 3-amino-1-propanesulfonic acid, *D*-glucosamine hydrochloride, aminomannitol, urea, human albumin, hydrazine, proteins, polyglycolamines, amino polyols such as HO—PEG—NH_2 or NH_2—PEG—NH_2, or compounds that contain acid groups, for example, PEG—*L*-asparagic acid, PEG—*L*-glutamic acid; PEG—*L*-DTPA and PEG—*L*-EDTA, where the molecular weight of the polyethylene glycol (PEG) is up to 100,000 daltons, preferably below 40,000 daltons.

The coupling agents listed under section I are condensed via their amino groups onto the formyl groups found on the surface of the microparticles made up of polymerized aldehydes and possibly surfactants.

Likewise bound via the formyl groups are the monomers listed under section IV, which can be polymerized with other monomers.

The acids and alcohols listed under sections I and II, by contrast, are coupled to the microparticles only after previous conversion of the aldehyde function.

By selecting suitable biomolecules or macromolecules bound via these coupling agents, for example, enzymes, dextrans, immunoglobulins, or monoclonal antibodies (see below), microparticles according to the invention are obtained which have a surprisingly high specificity for certain tissues and organs.

The microparticles according to the invention may contain diagnostically or therapeutically effective ingredients for the diagnosis and treatment of tumors, for example:

doxorubicin;

actinomycin;

magnetite;

mitomycin C; and

triamcinolone.

The microparticles according to the invention have the advantages described in the introduction, e.g., they can be produced simply and in high yield, their production can be scaled up, and they can be purified without difficulty.

Surprisingly, the microparticles according to the invention are better tolerated, especially in terms of cardiovascular effects (blood pressure, cardiac arrhythmia, and the like) and show no thrombogenic potential.

The particle-size distribution of these particles is narrow (monodisperse); at the same time, the size of the particles can be varied across a wide range depending on the concentrations selected for the starting materials (see below). By controlling the production conditions (for example, the pH value), it is also possible to vary the molecular weight in wide ranges.

A further advantage is that the reaction for synthesizing the microparticles can be initiated by many possible means, for example, anionic polymerization by changing the pH value, cationic polymerization with iron salts, for example, and radical polymerization using UV light and/or ionizing radiation.

The wide temperature range (−5 to +80°C) within which production of the microparticles is possible permits simple control of testing with optimal

yields for very different gases or readily volatile liquids.

The particles contain free aldehyde groups, which can be covalently bound with other molecules by chemical reactions. This feature makes it possible to alter the properties of the particle surface without influencing the echogenic properties. The colloidal stability can be influenced by selecting suitable coupling agents. In particular, the phenomenon of agglomeration frequently occurring for colloidal systems is prevented by this measure. This in turn is a great advantage for the development of a stable formulation.

In addition to influencing stability, there are ways to alter the surface of the particles in such a way that drug-targeting is possible. This is done by attaching suitable biomolecules or macromolecules (such as monoclonal antibodies) that bring about a high tissue- and organ-specificity [G. Gregoriadis and G. Poste, *The Targeting of Drugs*, Plenum Press, New York, 1988], or by influencing the surface properties of the particles by the adsorption of molecules (such as surfactants).

Depending on the choice of these molecules and the size of the microparticles, particle accumulation can be achieved in or on tumors or in the lungs, liver, spleen, and bone marrow, for example. Accumulation in the bone marrow, in particular, is achieved by coating small particles (< 100 nm) with Poloxamer 407, for example. If the particles are coated with Poloxamin 908, for example, these particles surmount the RES system, and they remain in the circulating blood (blood pool agent).

By coupling particles with antibodies, an accumulation of the particles in or on tumors can be achieved.

Active targeting can also be carried out with microparticles containing magnetite. The particles are accumulated in the desired sites in the intra-

vascular system by use of an externally applied magnetic field. This makes it possible to investigate flow conditions in blood vessels, for example.

Using particles that carry magnetite, it is also possible to create locally high temperatures by an externally applied alternating magnetic current. This can be used therapeutically, for example, in destroying tumors (hyperthermia therapy). In addition to an alternating magnetic field, an ultrasonic field can also be used. This, too, results in strong local temperature increases.

The microparticles according to the invention are produced by reacting an aqueous solution, containing 0-40%, and preferably 0.01-10% w/v, surfactant(s) and 0-10% w/v diagnostically or therapeutically effective ingredients and gases or readily volatile liquids, with stirring at a temperature of −5 to +80°C, and preferably 0-40°C, and at a pH value of 7-14, and preferably 9-13, for 1 minute to 10 hours, and preferably 1-10 hours, and possibly with the introduction of a gas, with copolymerizable aldehyde(s) up to a concentration of 0.1-50%, and preferably 3-20% w/v, based on the reaction mixture, as well as with copolymerizable additives in a concentration of 0-20%, and preferably 1-5% w/v, based on the reaction mixture, and with crosslinking agents at a concentration of 0-5%, and preferably 0.1-1% w/v, based on the reaction mixture, and then — possibly after purification — reacting the resulting microparticles with an aqueous solution that contains — based on the amount of aldehyde — up to equimolar amounts of a coupling agent as well as surfactants at a concentration of 0-20%, and preferably 0.01-10% w/v, based on the total volume, with stirring for up to 3 days, and preferably up to 2 days, at temperatures of 0-60°C, and preferably 5-30°C, and at a pH value of 3-9, and preferably 5-8, and — after purification if desired — these are possibly bound

to biomolecules or macromolecules.

The polymer—aldehyde particles obtained after the first reaction step have aldehyde groups on their surface. The reactions typical of aldehydes can be conducted with these aldehyde groups [R. C. Schulz, *Kolloidzeitschrift und Zeitschrift für Polymere*, Vol. 182, No. 1-2, p. 99, 1961; *Lehrbuch der organischen Chemie "Organikum"* (*Textbook of "Organic" Organic Chemistry*), VEB Verlag der Wissenschaften, Berlin, 1984]. As a result, it is possible for molecules that change the surface properties to be coupled to the particle surface.

Examples of possible reactions of the aldehyde groups are:

- reduction to an alcohol;
- oxidation to an acid;
- oximation;
- imine formation, possibly followed by hydrogenation and possibly subsequent *N*-alkylation;
- hydrazone formation, possibly followed by hydrogenation;
- mercaptalation;
- acetylation;
- NaOH-catalyzed dismutation (Cannizzaro reaction);
- aldol condensation.

The coupling of molecules that contain amino groups to the particles obtained in the first reaction step takes place by reaction with the aldehyde groups. Here, for example, the following experimental conditions are selected:

1,000 mg of polyacrolein particles is suspended in 50 mL of distilled water, then 5,000 mg of the reactant is added to this particle suspension which is stirred at room temperature. Stirring must continue according to the

reaction rate of the conversion; for slow reaction rates, this may be up to 48 hours. The particle suspension is then dialyzed (cutoff = 10,000 daltons).

If the substituents introduced by the reactions described above, for example, contain functional groups (possibly with intermediate protection), these can be converted, by methods known to those skilled in the art, to reactive groups suitable for coupling to biomolecules or macromolecules. Preferred groups of this type include the maleimidobenzoyl, 3-sulfomaleimidobenzoyl, 4-(maleimidomethyl)cyclohexylcarbonyl, 4-[3-sulfo(maleimidomethyl)]-cyclohexylcarbonyl, 4-(*p*-maleimidophenyl)butyryl, 3-(2-pyridyldithio)-propionyl, methacryloyl(pentamethylene)amido, bromoacetyl, iodoacetyl, 3-iodopropyl, 2-bromoethyl, 3-mercaptopropyl, 2-mercaptoethyl, phenylene isothiocyanate, 3-aminopropyl, benzyl ester, ethyl ester, *tert*-butyl ester, amino, C_1-C_6-alkylamino, aminocarbonyl; hydrazino, hydrazinocarbonyl, maleimido, methacrylamido, methacryloylhydrazinocarbonyl, maleimidamidocarbonyl, halogeno, mercapto, hydrazinotrimethylene hydrazinocarbonyl, aminodimethylene amidocarbonyl, bromocarbonyl, phenylene diazonium, isothiocyanate, semicarbazide, thiosemicarbazide, and isocyanate groups.

An amino group can be converted to an isothiocyanate group, for example, by methods found in the literature (for example, with thiophosgene in a two-phase system [S. Scharma, *Synthesis*, p. 803, 1978; D. K. Johnson, *J. Med. Chem.*, Vol. 32, p. 236, 1989].

By reacting an amino function with a halogen acetic acid halogenide, an α-halogen acetamide group can be formed [*JACS*, Vol. 90, p. 4,508, 1969; *Chem. Pharm. Bull.*, Vol. 29, No. 1, p. 128, 1981], which is as suitable as, for example, the isothiocyanate group used for coupling with biomolecules and macromolecules.

Examples of suitable substituents that can be introduced into a functional group that is suitable for binding to a macromolecule or biomolecule include hydroxyl and nitrobenzyl, hydroxyl and carboxyalkyl, and thioalkyl radicals with up to 20 carbon atoms. They are converted into the desired substituents (for example, with the amino, hydrazino, hydrazinocarbonyl, epoxide, anhydride, methacryloylhydrazinocarbonyl, maleimidamidocarbonyl, halogeno, halogenocarbonyl, mercapto, or isothiocyanate group as the functional group) by methods known to those skilled in the art and described in the literature [*Chem. Pharm. Bull.*, Vol. 33, p. 674, 1985; *Compendium of Org. Synthesis*, Vol. 1-5, Wiley and Sons, Inc; Houben-Weyl, *Methoden der organischen Chemie* (*Methods of Organic Chemistry*), Vol. VIII, Georg Thieme Verlag, Stuttgart; *J. Biochem.*, Vol. 92, p. 1,413, 1982]; in the case of the nitrobenzyl radical, a catalytic hydrogenation reaction [for example, as described in P. N. Rylander, *Catalytic Hydrogenation over Platinum Metals*, Academic Press, 1967] must first be carried out to form the aminobenzyl derivative.

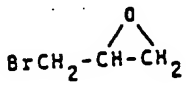
Examples of the conversion of hydroxyl or amino groups bound to aromatic or aliphatic radicals are reactions, carried out in suitable solvents such as tetrahydrofuran, dimethoxyethane, or dimethylsulfoxide, or two-phase aqueous systems such as water/dichloromethane, in the presence of an acid-capturer such as sodium hydroxide, sodium hydride, or alkali or alkaline-earth carbonates such as sodium, magnesium, potassium, or calcium carbonate, or poly(4-vinylpyridine) Reillex®, at temperatures between 0°C and the boiling point of the respective solvent, but preferably between 20°C and 60°C, with a substrate having the General Formula I:

$$Nf—L—Fu \qquad (I)$$

where Nf represents a "nucleofuge" such as Cl, Br, I, $CH_3C_6H_4SO_3$, or CF_3SO_3,

L represents an aliphatic, aromatic, arylaliphatic, branched, straight-chain, or cyclic hydrocarbon radical with up to 20 carbon atoms, and Fu represents the desired functional group, possibly in protected form [West German Offenlegungsschrift No. 3,417,413].

The following may be listed as examples of that can be represented by General Formula I: $Br(CH_2)_2NH_2$, $Br(CH_2)_3OH$, $BrCH_2COOCH_3$, $BrCH_2CO_2$-*tert*-Bu, $ClCH_2CONHNH_2$, $Br(CH_2)_4CO_2C_2H_5$, $BrCH_2COBr$, $BrCH_2CONH_2$, $ClCH_2COOC_2H_5$, $BrCH_2CONHNH_2$,



$CF_3SO_3(CH_2)_3Br$, $BrCH_2C{\equiv}CH$, $BrCH_2CH{=}CH_2$, $BrCH_2C_6H_4NCS$.

Conversions of carboxyl groups can be carried out, for example, by the carbodiimide method [Fieser, *Reagents for Organic Syntheses*, Vol. 10, p. 142], via a mixed aldehyde [*Org. Prep. Proc. Int.*, Vol. 7, p. 215, 1975], or via an activated ester [*Adv. Org. Chem.*, Part B, p. 472].

The resulting microparticles, carrying coupling agents, can also be bound to biomolecules or macromolecules that are known to accumulate especially in the organ or organ part to be investigated. Examples of such molecules are enzymes, hormones, polysaccharides such as dextrans or starches, porphyrins, bleomycins, insulin, prostaglandins, steroid hormones, amino sugars, amino acids, peptides such as polylysine, proteins (for example, immunoglobulins, monoclonal antibodies, and lectins), lipids (also in the form of liposomes), and nucleotides of the DNA or RNA type. Especially noteworthy are conjugates with albumins, such as human serum albumin, and antibodies, such as monoclonal antibodies, antibodies specific for tumor-associated antigens, or anti-myosin.

Instead of biological macromolecules, suitable synthetic polymers such as polyethyleneimines, polyamides, polyureas, polyethers such as polyethylene glycols, and polythioureas can be attached. The resulting pharmaceutical agents are suitable, for example, for use in the diagnosis of tumors and infarctions as well as tumor therapy. Monoclonal antibodies [for example, *Nature*, Vol. 256, p. 495, 1975] are superior to polyclonal antibodies in that they are specific for one antigenic determinant, have a defined binding affinity, are homogeneous (greatly facilitating their pure production), and can be produced in large quantities in cell cultures. Suitable, for example, for visualizing tumors are monoclonal antibodies or their fragments Fab and $F(ab')_2$, which are specific, for example, for human tumors of the gastrointestinal tract, the breast, the liver, the bladder, the gonads, and melanomas [*Cancer Treatment Reports*, Vol. 68, p. 317, 1984; *Bio Sci*, Vol. 34, p. 140, 1984], or are directed against carcinoembryonic antigen (CEA), human chorionic gonadotropin (β-HCG), or other tumor-resistant antigens, such as glycoproteins [*New Engl. J. Med.*, Vol. 298, p. 1,384, 1984; U.S. Patent No. 4,331,647]. Other suitable substances include anti-myosin, anti-insulin, and anti-fibrin antibodies [U.S. Patent No. 4,036,945].

Colon carcinomas can be diagnostically detected using microparticle conjugates with the antibody 17-1A (Centocor, USA).

In the case of antibody conjugates, the binding of the antibody to the microparticle must not be permitted to lead to a loss or reduction in the antibody-to-antigen binding affinity and binding specificity. This can occur either by binding to the carbohydrate portion in the Fc part of the glycoprotein or in the Fab or $F(ab')_2$ fragments, or by binding to sulfur atoms of the antibody or the antibody fragments.

In the first case, it is necessary to first oxidatively cleave the sugar units to create formyl groups that are capable of coupling. This oxidation can be carried out by chemical methods with oxidizing agents, such as periodic acid, sodium metaperiodate, or potassium metaperiodate, by methods found in the literature [for example, *J. Histochem. and Cytochem.*, Vol. 22, p. 1,084, 1974], in aqueous solution in concentrations of 1-100, and preferably 1-20 mg/mL, and a concentration of the oxidizing agent of 0.001-10 mmoles, and preferably 1-10 mmoles, in a pH range of ca. 4-8, at a temperature of 0-37°C and with a reaction time of 15 minutes to 24 hours. The oxidation can also be carried out by enzymatic methods, for example, by using galactose oxidase at an enzyme concentration of 10-100 units/mL, a substrate concentration of 1-20 mg/mL, a pH value of 5-8, a reaction time of 1-8 hours, and a temperature of 20-40°C [see, for example, *J. Biol. Chem.*, Vol. 234, p. 445, 1959].

Microparticles with suitable functional groups, for example, hydrazine, hydrazide, hydroxylamine, phenylhydrazine, semicarbazide, and thiosemicarbazide groups, are bound to the aldehydes created by the oxidation reaction, by reaction at 0-37°C, a reaction time of 1-65 hours, a pH value of 5.5-8, an antibody concentration of 0.5-20 mg/mL, and a molar ratio of the complexing agent to the antibody aldehyde of 1:1 to 1,000:1. The subsequent stabilization of the conjugate occurs by reduction of the double bond, for example, with sodium borohydride or sodium cyanoborohydride; the reducing agent is used in an excess of 10-100 times [see, for example, *J. Biol. Chem.*, Vol. 254, p. 4,359, 1979].

The second possibility for forming antibody conjugates is based on a sparing reduction of the disulfide linkages of the immunoglobulin molecule; in this process the more sensitive disulfide linkages between the H chains of the

antibody molecule are broken, while the S—S bonds of the antigen-binding region remain intact, so that practically no reduction of the binding affinity and binding specificity of the antibody occurs [*Biochem.*, Vol. 18, p. 2,226, 1979; *Handbook of Experimental Immunology*, Second Edition, Vol. 1, Chapter 10, Blackwell Scientific Publications, London, 1973]. These free sulfhydryl groups of the inter-H chain regions are then reacted with suitable functional groups of the microparticles at 0-37°C, a pH value of ca. 4-7, and a reaction time of 3-72 hours, to form a covalent bond that does not affect the antigen-binding region of the antibody. The following may be cited as examples of suitable reactive groups: halogen alkyl, halogen acetyl, *p*-mercuribenzoate, isothiocyanate, thiol, and epoxide groups, as well as groups that can be subjected to a Michael addition reaction, for example, maleinimides and methacrylo groups [see, for example, *J. Amer. Chem. Soc.*, Vol. 101, p. 3,097, 1979].

To attach the antibody fragments to the microparticles, a number of suitable, and often commercially available, bifunctional "linkers" also exist [see, for example, Pierce, *Handbook and General Catalogue*, 1986], which can react with both the SH groups of the fragments and the amino or hydrazino groups of the microparticles. The following may be cited as examples:

m-maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS);

m-maleimidobenzoyl-*N*-sulfosuccinimide ester (sulfo-MBS);

N-succinimidyl[4-(iodacetyl)amino]benzoic acid ester (SIAB);

succinimidyl-4-(*N*-maleimidomethyl)-1-cyclohexane carboxylic acid ester (SMCC);

succinimidyl-4-(*p*-maleimidomethyl)butyric acid ester (SMPB);

N-succinimidyl-3-(2-pyridyldithio)propionic acid ester (SDPD);

4-[3-(2,5-dioxo-3-pyrrolinyl)propionyloxy]-3-oxo-2,5-diphenyl-2,3-dihydrothiophene-1,1-dioxide;

acetylalanylleucylalanylamino-*p*-benzyl;

acetamido-*p*-thioureidobenzyl.

Bonds other than covalent bonds can also be used for coupling to the biomolecule or macromolecule; both ionic bonds and van der Waals and hydrogen bridge bonds can contribute to the bond (for example, avidin—biotin, antibody—antigen) in varying amounts and strengths (key—lock principle). Inclusion compounds (host-guest) enclosing smaller complexes in larger cavitands in the macromolecule are also possible.

The coupling principle consists of first producing a bifunctional macromolecule, either by fusing an antibody-hybridoma directed against a tumor antigen with a second antibody-hybridoma directed against the microparticle according to the invention, or by joining the two antibodies chemically via a linker [for example, as described in *J. Amer. Chem. Soc.*, Vol. 101, p. 3,097, 1979], or by binding the antibody directed against the tumor antigen to avidin (or biotin), possibly via a linker [D. J. Hnatowich et al., *J. Nucl. Med.*, Vol. 28, p. 1,294, 1987]. In place of the antibodies, their corresponding F(ab) or $F(ab')_2$ fragments can also be used. For pharmaceutical applications, the bifunctional macromolecule is injected first, which then accumulates at the target site, and after a certain amount of time the microparticles according to the invention (possibly bound to biotin (or avidin)) are injected, which are coupled *in vivo* on the target site where they can exert their diagnostic or therapeutic action. Furthermore, other coupling methods can also be used, such as the "reversible radiolabeling" described in *Protein Tailoring Food Med. Uses* [Am. Chem. Soc. Symp., p. 349, 1985].

So-called solid-phase coupling provides an especially simple method for producing antibody conjugates or antibody fragment conjugates. The antibody is coupled to a stationary phase (for example, an ion exchanger), which is located in a glass column, for example. By successively rinsing the column with a solution suitable for generating aldehyde groups, washing, rinsing with a solution of the functionalized microparticles, washing, and finally eluting the conjugate, very high conjugate yields are obtained.

This method permits the automatic and continuous production of any amount of conjugates.

Other coupling steps can also be carried out in this manner.

For example, fragment conjugates can be produced by the sequence of papain reduction, then bifunctional linker, then functionalized microparticle.

The resulting compounds are then preferably purified by chromatography.

Particles measuring 0.04-100 μm, and preferably 0.1-40 μm, can be produced. The size of the particles can be considerably influenced by varying the initial concentration of the monomer, the surfactant, and the pH value.

Examples of the production of particles of specific sizes:

Example 1

Acrolein concentration 10% (w/v); surfactant concentration 1.5% (w/v); pH 10.0; and temperature = 4°C.

If these conditions are selected, particles with an average diameter of 750 nm can be obtained.

Example 2

Acrolein concentration 20% (w/v); surfactant concentration 0.2% (w/v); pH 10.0; and temperature = 2°C.

Under these conditions, particles with an average diameter of 40 μm can be obtained.

Example 3

Under the same conditions as in Example 2, but with an acrolein concentration of 10% (w/v), particles with an average diameter of 8 μm can be obtained.

Example 4

Acrolein concentration 10% (w/v); surfactant concentration 0.5% (w/v); pH 11.0. Average particle size = 560 nm.

Example 5

Under the same conditions as in Example 4, but with a pH of 9.0, particles with an average diameter of 3.2 μm can be obtained.

Polyglutaraldehyde sodium hydrogen sulfite adduct (PGL) is used as the surfactant.

Instead of the polyglutaraldehyde sodium hydrogen sulfite adduct (PGL), polyacrolein sodium hydrogen sulfite adduct (PAC-SO_3) can also be used without an observable effect on the particle size.

SYNTHESIS OF PGL

A 25% aqueous solution of glutaraldehyde is purified over activated charcoal, then N_2 is directed through the aqueous solution to drive out the O_2. Also, a buffer solution (phosphate buffer, 1 molar) is used to adjust the pH to 11. The buffer solution is also freed of O_2 by introducing N_2. The buffer solution and the glutaraldehyde solution are combined and polymerized in an N_2 atmosphere for 72 hours, then the polymer is filtered and washed with acetone and water, and dried in a vacuum-drying oven at 45°C. 5 g of polyglutaraldehyde is dissolved in 30 mL of H_2O that contains 12.5 g of $NaHSO_3$. The solution is dialyzed against distilled H_2O then lyophilized.

The particles according to the invention can be suspended in aqueous solutions with no particle aggregation. To produce a galenic formulation that can be administered parenterally, aqueous solutions are used that contain isotonizing additives such as sodium chloride, sugar alcohols (mannitol, sorbitol, xylitol, and so forth), or sugar (glucose, fructose). To adjust the pH value, buffers such as tromethamine/HCl, citric acid/NaOH, and so forth can be selected.

SYNTHESIS OF PAC-SO_3

A. 100 mL of distilled water is placed in a three-neck flask equipped with dropping funnel and stirrer and the O_2 is driven out with nitrogen. 1.829 g of $K_2S_2O_8$ is then added to this water and dissolved. After the $K_2S_2O_8$ has completely dissolved, 20 mL of freshly distilled acrolein is added, then 1.14 g of $AgNO_3$ dissolved in 5 mL of water is added and the mixture is stirred for 2 hours as it polymerizes. The precipitated polymer is

filtered off, washed several times with water, and resuspended for 1 hour in 1 L of water in which 1.63 g of sodium thiosulfate has been dissolved, in order to remove the silver ions. The polymer is filtered off, dried in a vacuum-drying oven at 45°C, then coarsely ground in a mortar. 10 g of ground polymer is dissolved in 100 mL of sodium hydrogen sulfite (37%), then the solution is dialyzed against distilled water (cutoff = 5,000 daltons), and the dialyzate is used as a surfactant in the preparation of the polyacrolein microparticles.

B. 100 mL of distilled water is placed in a round-bottom flask and 20 mL of freshly distilled acrolein is added with stirring. The pH of the reaction mixture is then adjusted to 10.5 with 2 *N* NaOH and the reaction mixture is stirred for 2 hours as it polymerizes. The precipitated polymer is filtered off, washed several times with water, and dried in a vacuum-drying oven at 45°C. 10 g of the polymer is then dissolved in 100 mL of $NaHSO_3$ solution (37%), the solution is dialyzed against distilled water (cutoff = 5,000 daltons), and the residue is used as a surfactant in the preparation of the polyacrolein microparticles.

Example 1

100 mL of the formulation contains 100 mg of particles, 2.4 mg of trometamol, enough HCl for a pH of 7.4, 5,500 mg of mannitol, and enough water to make 100 mL.

Example 2

100 mL of the formulation contains 50 mg of particles, 860 mg of sodium

chloride, and enough water to make 100 mL.

Floating of the particles can be prevented by adjusting the average density of the particles to that of the surrounding vehicle.

This can be achieved by adding substances of greater density (radiological contrast media, magnetite). This possibility is especially useful for particles with a low polyaldehyde content.

The pharmaceutical agents according to the invention contain from 0.1 μg to 100 mg of microparticles per mL, and preferably from 10 μg to 1 mg of microparticles per mL of galenic formulation. These agents are generally administered in doses of 0.01-10 mL/kg, and preferably 0.1-1 mL/kg of body weight. They are intended for enteral and parenteral administration.

For use in hyperthermia therapy, the pharmaceutical agents according to the invention are generally used in doses of 0.001-10 mg, and preferably 0.01-1 mg per g of tumor.

The examples below are intended to describe the invention, but the present invention is not limited to these examples.

METHODS FOR PRODUCING THE CONTRAST MEDIUM

Reaction Step 1

(A) An aqueous solution containing a surfactant (0.01-5% w/v) is stirred while it is cooled to 0°C. At the same time, a gas is directed through the solution. The pH of the solution is adjusted with NaOH to the desired pH value (preferably 9-13). The monomer or monomer mixture is then added to this solution and after 30 minutes, the stirring speed is reduced. After 1 hour, the reaction mixture is diluted with the aqueous surfactant-containing solution described above and the stirring speed is reduced still further. After 4

hours, the precipitated microparticles that contain no gas are removed by decanting the remaining suspension, and discarded. The decanted suspension is dialyzed to remove any residual monomers from the contrast medium. Yield = 80-90%.

(B) An aqueous solution containing the selected amount of surfactant and monomer is cooled to 0°C. At the same time, the selected gas is directed through the solution as it is stirred. The pH of the solution is then adjusted with NaOH to preferably 9-13. After 1 hour, the reaction mixture is diluted. After 3-4 hours, the suspension that contains the microparticles is separated from the precipitated polymer, which is discarded. The suspension is then purified by dialysis. Yield = 80-90%.

Example 1

91 mL of a 0.5% aqueous surfactant solution is placed in a flask and the pH is adjusted to 11 with 0.2 *N* NaOH solution. N_2 is directed through the solution. The 0.5% surfactant solution is cooled to 0°C and 9.5 mL of freshly distilled acrolein is added drop by drop. After 1 hour, an additional 100 mL of 0.5% surfactant solution is added to the reaction mixture. After 3 hours, the suspension that contains the microparticles is decanted from the precipitated polymers and purified by dialysis.

Example 2

82 mL of an 0.08% aqueous surfactant solution is placed in a flask. The solution is cooled to 0°C and 18 mL of freshly distilled acrolein is added. Argon is directed through the solution while it is stirred. After 1 hour, the

pH value of the solution is adjusted to 12 with 0.2 *N* NaOH solution. After 2 hours, 100 mL of the 0.08% surfactant solution is added. After 3 hours, the suspension is decanted and dialyzed.

Example 3

70 mL of an 0.08% aqueous surfactant solution containing 10% dimethylformamide is placed in a flask and the pH is adjusted to 11.5 with 0.2 *N* NaOH solution. The solution is then cooled to 0°C while N_2 is directed through it. 30 mL of freshly distilled acrolein is added to this solution drop by drop. After 1 hour, 100 mL of the 0.08% surfactant solution is added, and after 4 hours, the suspension is separated from the precipitated polymers and purified.

Example 4

91 mL of a 0.5% aqueous surfactant solution containing 5% magnetite is cooled to 0°C in a flask and the pH is adjusted to 12 with 0.2 *N* NaOH solution. N_2 is directed through the solution. 9 mL of freshly distilled acrolein which has been cooled to 0°C is then added. After 1 hour, 100 mL of the 0.5% surfactant solution is added to the reaction mixture. The suspension that contains the microparticles is decanted from the precipitated polymers and dialyzed.

Example 5

91 mL of a 0.5% aqueous surfactant solution is placed in a flask and the pH is adjusted to 12 with 0.2 *N* NaOH solution. The solution is cooled to 0°C

and argon is directed through it. 9 mL of freshly distilled acrolein containing 5% butylcyanoacrylate is then added to this solution drop by drop. After 1 hour, an additional 100 mL of the 0.5% surfactant solution is added. The suspension is then separated from the sediment and purified.

Example 6

91 mL of an 0.08% aqueous surfactant solution is placed in a flask and the pH is adjusted to 10.5 with 0.2 *N* NaOH solution. The solution is cooled to 0°C and N_2 is directed through it. 9 mL of freshly distilled acrolein that contains 20% α-methylacrolein is added to the solution drop by drop. After 1 hour, an additional 100 mL of the 0.08% surfactant solution is added. After 2 hours, the microsphere suspension is separated from the sediment and purified.

Example 7

91 mL of an 0.08% aqueous surfactant solution containing 25% isopentane is placed in a flask and cooled to 0°C. 9 mL of freshly distilled acrolein is then stirred into the solution, and after 2 hours the reaction mixture is filtered. The microparticles are purified by washing with water and then are resuspended in water. Surfactant solution: polyglutaraldehyde sodium hydrogen sulfite adduct.

Reaction Step 2

A distilled water suspension of polyacrolein microparticles is adjusted to a pH 6.5 with 0.01 *N* HCl solution. An excess of amine-containing ligands

is stirred into this suspension at room temperature. The pH of this latter solution is previously adjusted to 8 with 0.01 *N* NaOH solution.

The reaction mixture is then stirred at room temperature for up to 48 hours, depending on the reaction rate. To remove the excess amine-containing ligands, the product is dialyzed against water.

If necessary, the resulting Schiff bases are reduced to the amines by adding reducing agents.

Example 6 [sic; should be 8 -- Tr. Ed.]

1,000 mg of polyacrolein microparticles from Example 1 is resuspended in 50 mL of water, then 1,000 mg 3-amino-1-propanesulfonic acid is added and the suspension is stirred for 48 hours at room temperature. The suspension is then dialyzed against water.

Next, the product is mixed with 150 mg of $NaBH_3CN$ and stirred for 24 hours while keeping the pH at 7.5. The resulting suspension is then dialyzed against water.

If necessary, the amine can be alkylated or acetylated with chloroacetic acid, acetic anhydride, or diglycolic acid anhydride.

Example 9

1,000 mg of polyacrolein microparticles from Example 2 is resuspended in 50 mL water, then 1,000 mg of 3-aminopropane phosphate is added and the suspension is stirred for 48 hours at room temperature. The suspension is then dialyzed against water.

The resulting product is mixed with 150 mg of $NaBH_3CN$ and stirred for 24

hours while keeping the pH at 7.5, and the suspension that forms is dialyzed against water.

If necessary, the amine can be alkylated or acetylated with chloroacetic acid, acetic anhydride, or diglycolic acid anhydride.

Example 10

1,000 mg of polyacrolein microparticles from Example 3 is resuspended in 50 mL of water, then 1,000 mg of 8-aminooctanoic acid is added and the suspension is stirred for 24 hours at room temperature. The suspension is then dialyzed against water.

The resulting product is mixed with 150 mg of $NaBH_3CN$ and stirred for 24 hours while keeping the pH at 7.5 and the suspension that forms is dialyzed against water.

If necessary, the amine can be alkylated or acetylated with chloroacetic acid, acetic anhydride, or diglycolic acid anhydride.

Example 11

1,000 mg of polyacrolein microparticles from Example 4 is resuspended in 50 mL of water, then 1,000 mg 5-aminopentanoic acid is added and the suspension is stirred for 36 hours at room temperature. The suspension is then dialyzed against water.

The resulting product is mixed with 150 mg of BH_3CN and stirred for 24 hours while keeping the pH at 7.5 and the suspension that forms is dialyzed against water.

If necessary, the amine can be alkylated or acetylated with chloroacetic acid, acetic anhydride, or diglycolic acid anhydride.

Example 12

1,000 mg of polyacrolein microparticles from Example 5 is resuspended in 50 mL of water, then 1,000 mg of *D*-glucosamine hydrochloride is added and the suspension is stirred for 30 hours at room temperature. The suspension is then dialyzed against water.

The resulting product is mixed with 150 mg of $NaBH_3CN$ and stirred for 24 hours while keeping the pH at 7.5 and the suspension that forms is dialyzed against water.

If necessary, the amine can be alkylated or acetylated with chloroacetic acid, acetic anhydride, or diglycolic acid anhydride.

Example 13

1,000 mg of polyacrolein microparticles from Example 6 is resuspended in 50 mL of water, then 1,000 mg hexamethylenediamine is added and the suspension is stirred for 24 hours at room temperature. The suspension is then dialyzed against water.

The resulting product is mixed with 150 mg of $NaBH_3CN$ and stirred for 24 hours while keeping the pH at 7.5 and the suspension that forms is dialyzed against water.

If necessary, the amine can be alkylated or acetylated with chloroacetic acid, acetic anhydride, or diglycolic acid anhydride.

Example 14

1,000 mg of polyacrolein microparticles from Example 7 is resuspended in 50 mL of water, then 1,000 mg polylysin (molecular weight = 32,600 daltons) is added and the suspension is stirred for 30 hours at room temperature. The suspension is then washed with water.

Example 15

100 mg of polyacrolein microparticles from Example 5 is resuspended in 2.5 mL of water, then 250 of mg human serum albumin dissolved in 2.5 mL of water is added and the suspension is stirred for 8 hours at room temperature. The suspension is then dialyzed against distilled water (cutoff = 100,000).

Example 16

1,000 mg of polyacrolein microparticles from Example 4 is resuspended in 50 mL of water, then 1,000 mg of (2-diethylamino)ethylamine is added and the suspension is stirred for 20 hours at room temperature. The suspension is then dialyzed against water.

Example 17

300 mg of polyacrolein microparticles from Example 1 is resuspended in 7.5 mL of distilled water, then 750 mg of 3-amino-1-propanesulfonic acid dissolved in 7.5 mL of water is added and the suspension is stirred for 24 hours at room temperature. The suspension is then dialyzed against distilled water (cutoff = 1,000 daltons).

Example 18

200 mg of polyacrolein microparticles from Example 7 is resuspended in 5 mL of distilled water, then 500 mg of lysin dissolved in 5 mL of water is added and the suspension is stirred for 24 hours at room temperature. The suspension is then dialyzed against distilled water (cutoff = 5,000 daltons).

In Vitro Experiments

In vitro experiments were conducted by measuring the backscattering of the echo amplitudes of suspensions of selected microparticles according to the invention; the results confirmed their very good acoustic properties.

The *in vitro* experiments will now be described with reference to the attached figures.

The measuring apparatus consisted of an ultrasonic transmitter combined with an ultrasonic receiver and a measurement cell containing the sample. To measure the acoustic properties of the sample, an ultrasonic pulse is transmitted which scatters on the glass wall of the cell, passes through the sample, and then, if the sample is not echogenic, scatters on the rear wall of the cell. The reflected ultrasonic pulse is picked up by the receiver and represented as a change in amplitude (see figures).

Figure 1 shows the pulse-reflected behavior of water (as an example of a non-echogenic blank). Clearly recognizable are the pulse-reflected amplitudes of the front wall of the cell (at 3 μs) and the back wall (at ca. 16 μs).

If an echogenic sample is measured, a pulse-reflected recording like that shown in Figures 2-4 occurs. No reflection from the cell wall is received, because interaction with the echogenic sample causes the ultrasound to dissi-

pate, or alters it in such a way that it is no longer reflected back to the receiver.

The pulse-reflected amplitudes of aqueous particle suspensions from Example 8 (Figure 2), Example 11 (Figure 3), and Example 15 (Figure 4) were recorded, in each case at a concentration of 0.5 mg/mL.

In Vivo Experiments

To conduct an echocardiographic examination of a dog (beagle) weighing about 10 kg, the contrast media according to the invention were used as follows: 1 mL of the solution, containing 40 μg/mL of particles coupled with albumin (Example 15) in 5% glucose solution, was taken from the vial containing the ready-to-use suspension. This contrast medium was injected into the saphenous vein of the caudal ramus via a three-way cock open on all sides, at an injection rate of at least 1 mL/s, but more favorably at a rate of 3 mL/s, followed by a second injection of 5 mL of physiological saline solution (0.9%). The secondary injection was administered to keep the contrast medium bolus intact for as long as possible. Before the injection (Figure 5), an "apical four-chamber view" of the test animal's heart was prepared for echocardiography using an ordinary commercial sound head [= *transducer*] attached to the chest wall (transthoracic recording) with a clamp. Before, during, and after the injection, the sonogram was displayed on the monitor of the ultrasonic examination apparatus and documented as necessary on videotape or with a video printer. This test arrangement corresponds to the state of the art and is known to those skilled in the art.

When theultrasonic contrast medium reaches the right heart, the contrast effect can be monitored in color Doppler, two-dimensional, or M-mode ultrason-

ography. Contrast is first obtained in the blood in the right atrium, then in the right ventricle, and finally in the pulmonary artery. The filling process is essentially homogeneous and is maintained long enough for the diagnostic examination to be done. While the cavities of the right heart fill again with uncontrasted blood (there is a decrease and disappearance of the cloud of echoes from the heart cavities), the contrast medium, after (transcapillary) passage through the lungs, appears in the pulmonary veins, then homogeneously fills the left atrium, the left ventricle, and the subsequent high-pressure system. The contrast effect in the left heart cavities lasts longer than on the right side of the heart. Once the contrast of the left heart cavities is obtained, further contrast is obtained for other organs through which the circulation passes.

Figure 6 shows the contrast medium filling the left ventricle.

The use of the ultrasonic contrast medium according to the invention is not limited to the visualization of the blood flow in the vascular system; contrast can be obtained for other body cavities just as easily. As a result of the circulatory visualization, other organs can also be successfully examined with these contrast media.

CLAIM(S)

1. Microparticles consisting of biodegradable polymers, characterized in that they are made from polymerizable aldehydes, possibly containing copolymerizable additives and/or crosslinking agents, possibly surfactants or surfactant mixtures, gases and/or readily volatile liquids in free or bound form, coupling agents, possibly biomolecules or macromolecules bound via these cou-

pling agents, and possibly diagnostically or therapeutically effective ingredients.

2. Pharmaceutical agents, characterized in that they contain microparticles as described in Claim 1 and that possibly contain additives of the kind typically used in galenicals.

3. Microparticles as described in Claim 1, characterized in that they are used in ultrasonic diagnosis.

4. Microparticles as described in Claim 1, characterized in that they are used to product partial, externally induced hyperthermia.

5. Microparticles as described in Claim 1, characterized in that they can be controlled by an external magnetic field.

6. Microparticles as described in Claim 1, characterized in that the polymerizable aldehydes are selected from the following:

I. α,β-unsaturated aldehydes, for example:

acrolein;

crotonaldehyde;

propionaldehyde;

II. α-substituted acrolein derivatives, for example:

α-methylacrolein;

α-chloroacrolein;

α-phenylacrolein;

α-ethylacrolein;

α-isopropylacrolein;

α-*N*-butylacrolein;

α-*N*-propylacrolein;

III. Dialdehydes, for example:

glutaraldehyde, succinaldehyde or their derivatives or mixtures of these with copolymerizable additives, for example:

α-substituted acroleins;

β-substituted acroleins;

ethylcyanoacrylates;

methylcyanoacrylates;

butylcyanoacrylates;

hexylcyanoacrylates;

methyl methacrylates;

vinyl alcohols;

acrylic acids;

methacrylic acids;

acrylic acid chlorides;

methacrylic acid chlorides;

acrylonitrile;

methacrylonitriles;

acrylamides;

substituted acrylamides;

hydroxymethyl methacrylates;

mesityl oxide;

2-dimethylaminoethyl methacrylates;

vinylpyridines; and

N-vinyl-2-pyrrolidinone.

Acrolein and glutaraldehyde are the constituents of first choice.

7. Microparticles as described in Claim 1, characterized in that the

surfactants can be selected from ionogenic or non-ionogenic surface-active substances (surfactants), for example:

polyethylene oxide;

polyoxyethylene polyoxypropylenes such as Pluronic® F 68, Pluronic® F 108, Pluronic® F 127;

polyethylene glycol, Poloxamin 908, Polaxamer 407;

carboxylic acid salts, for exampl

polyoxyethylene fatty acid esters, for example:

polyoxyethylene stearate;

sodium dioctyl sulfosuccinate;

polyglutaraldehyde sodium hydrogen sulfite adducts;

polyacrolein sodium hydrogen sulfite adducts; and

polyvinylsulfonic acid.

8. Microparticles as described in Claim 1, characterized in that the gases or readily volatile liquids include:

ammonia;

air;

noble gases (helium, neon, argon, xenon, and krypton);

sulfur halides, for example, sulfur hexafluoride;

nitrogen;

carbon oxides;

oxygen;

hydrogen;

hydrocarbons or their mixtures, for example:

methane;

ethane;

propane;

butane;

pentane;

neopentane;

isopentane;

cyclopentane;

ethylene;

propylene;

acetylene;

3,3-dimethyl-1-butyne;

2,3-pentadiene;

2-methyl-2-butene;

2-methyl-1,3-butadiene;

2-butyne;

2-methyl-1-butene;

3-methyl-1-butene;

halogenated hydrocarbons or mixtures, for example:

methylene chloride;

1,1-dichloroethylene;

isopropyl chloride;

dibromodifluoromethane;

bromomethane;

ethers, for example: dimethyl ether, diethyl ether, or fluorinated ether;

or compounds such as:

dimethylaminoacetone;

propylene oxide;

N-ethylmethylamine;

N-ethyldimethylamine; and

furan.

9. Microparticles as described in Claim 1, characterized in that the coupling agents include:

I. Compounds that contain amino groups, for example;

hydroxylamine;

butylamine;

allylamine;

ethanolamine;

trishydroxymethylaminomethane;

3-amino-1-propanesulfonic acid;

5-aminopentanoic acid;

8-aminooctanoic acid;

D-glucosamine hydrochloride;

aminogalactose;

aminosorbitol;

aminomannitol;

diethylaminoethylamine;

anilines;

sulfonyl acid amide;

choline;

N-methylglucamine;

piperazine;

1,6-hexanediamine;

urea;

hydrazine;

glycine;

alanine;

lysine;

serine;

valine;

leucine;

peptides;

proteins;

albumin;

human serum albumin;

polylysine;

gelatins;

polyglycol amines;

amino polyols;

dextran sulfates with amino groups;

N-aminopolyethylene glycol ($HO-PEG-NH_2$);

N,*N*'-diaminopolyethylene glycol ($NH_2-PEG-NH_2$);

antibodies; and

immunoglobulins.

II. Compounds that contain acid groups, for example:

carboxylic acids;

acetic acid;

propionic acid;

butyric acid;

valeric acid;

caproic acid;

caprylic acid;

capric acid;

lauric acid;

myristic acid;

palmitic acid;

stearic acid;

oleic acid;

linoleic acid;

linolenic acid;

cyclohexane carboxylic acid;

phenylacetic acid;

benzoylacetic acid;

chlorobenzoic acid;

bromobenzoic acid;

nitrobenzoic acid;

ortho-phthalic acid;

meta-phthalic acid;

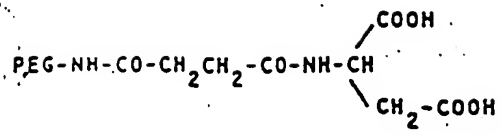
para-phthalic acid;

salicylic acid;

hydroxybenzoic acid;

aminobenzoic acid;

methoxybenzoic acid;



(PEG—*L*-asparagic acid);

PEG—*L*-glutamic acid;

PEG—*L*-DTPA;

PEG—*L*-EDTA;

III. Compounds that contain hydroxyl groups, for example:

alcohols;

methanol;

ethanol;

propanol;

butanol;

pentanol;

hexanol;

heptanol;

octanol;

decanol;

dodecanol;

tetradecanol;

hexadecanol;

octadecanol;

isopropyl alcohol;

isobutyl alcohol;

isopentyl aclohol;

cyclopentanol;

cyclohexanol;

crotyl alcohol;

benzyl alcohol;

phenyl alcohol;

diphenylmethanol;

triphenylmethanol;

cinnamic alcohol;

ethylene glycol;

1,3-propanediol;

glycerol;

pentaerythritol;

IV. Polymerizable substances, such as

α,β-unsaturated aldehydes, for example:

acrolein;

crotonaldehyde;

propynaldehyde;

α-substituted acrolein derivatives, for example:

α-methylacrolein;

α-chloroacrolein;

α-phenylacrolein;

α-ethylacrolein;

α-isopropylacrolein;

α-butylacrolein;

α-propylacrolein;

dialdehydes, for example:

glutaraldehyde, succinaldehyde, or their derivatives, or mixtures of

these with copolymerizable additives, for example:

α-substituted acroleins;

β-substituted acroleins;

ethylcyanoacrylates;

methylcyanoacrylates;

butyl acrylates;

hexylcyanoacrylates;

methyl methacrylates;

vinyl alcohols;

acrylic acids;

methacrylic acids;

acrylic acid chlorides;

acrylonitrile;

methacrylonitriles;

acrylamides;

substituted acrylamides;

hydroxymethyl methacrylates;

mesityl oxide;

2-dimethylaminoethyl methacrylates;

vinylpyridines; and

N-vinyl-2-pyrrolidinone.

10. Microparticles as described in Claim 1, characterized in that they contain organ- or tissue-specific compounds, for example, monoclonal antibodies, as the biomolecules or macromolecules.

11. Microparticles as described in Claim 1, characterized in that they

contain diagnostically or therapeutically effective ingredients for the diagnosis and treatment of tumors, for example:

doxorubicin;

actinomycin;

magnetite;

mitomycin C; and

triamcinolone.

12. Method for the preparation of microparticles as described in Claim 1, characterized in that an aqueous solution, containing 0-40%, and preferably 0.01-10% w/v, surfactant(s) and 0-10% w/v diagnostically or therapeutically effective ingredients and gases or readily volatile liquids are reacted with stirring at a temperature of −5 to +80°C, and preferably 0-40°C, and at a pH value of 7-14, and preferably 9-13, for 1 minute to 10 hours, and preferably 1-10 hours, and possibly with the introduction of a gas, with copolymerizable aldehyde(s) up to a concentration of 0.1-50%, and preferably 3-20% w/v, based on the reaction mixture, as well as with copolymerizable additives in a concentration of 0-20%, and preferably 1-5% w/v, based on the reaction mixture, and with crosslinking agents at a concentration of 0-5%, and preferably 0.1-1% w/v, based on the reaction mixture, and then — possibly after purification — reacting the resulting microparticles with an aqueous solution that contains — based on the amount of aldehyde — up to equimolar amounts of a coupling agent as well as surfactants at a concentration of 0-20%, and preferably 0.01-10% w/v, based on the total volume, with stirring for up to 3 days, and preferably up to 2 days, at temperatures of 0-60°C, and preferably 5-30°C, and at a pH value of 3-9, and preferably 5-8, and — after purification if desired — these are possibly bound to biomolecules or macromolecules.

13. Method for preparation of the pharmaceutical agents described in Claim 2, characterized in that the microparticles dissolved or suspended in water, possibly with the additives typically used in galenicals, are processed into a form suitable for enteral or parenteral administration.

14. Microparticles as described in Claim 1, characterized in that they have a diameter of 0.1-40 μm.

15. Pharmaceutical agents as described in Claim 2, characterized in that the concentration of the microparticles is from 1 μg to 100 mg per mL, and preferably 10 μg to 1 mg per mL, of the galenic formulation.

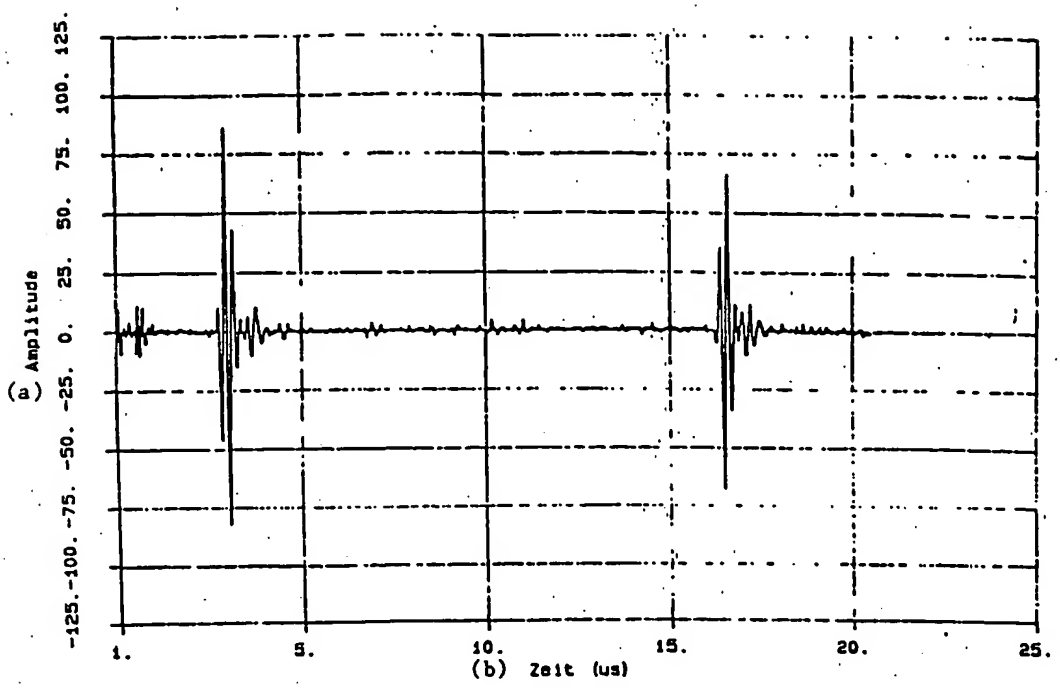


Figure 1. KEY: (a) amplitude; and (b) time, μs.

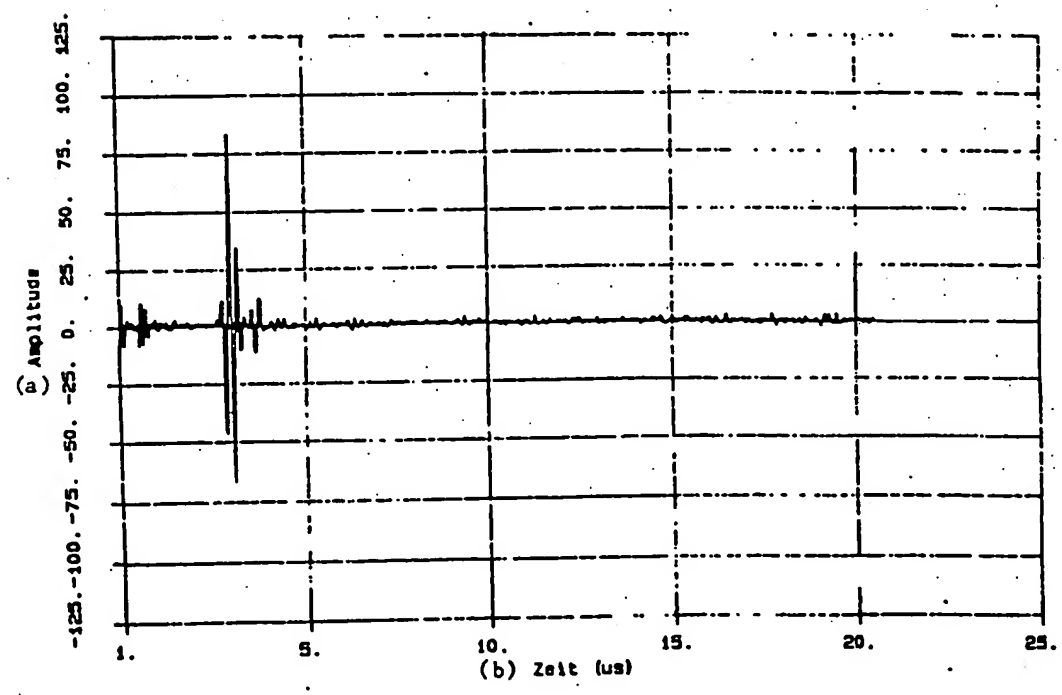


Figure 2. KEY: (a) amplitude; and (b) time, μs.

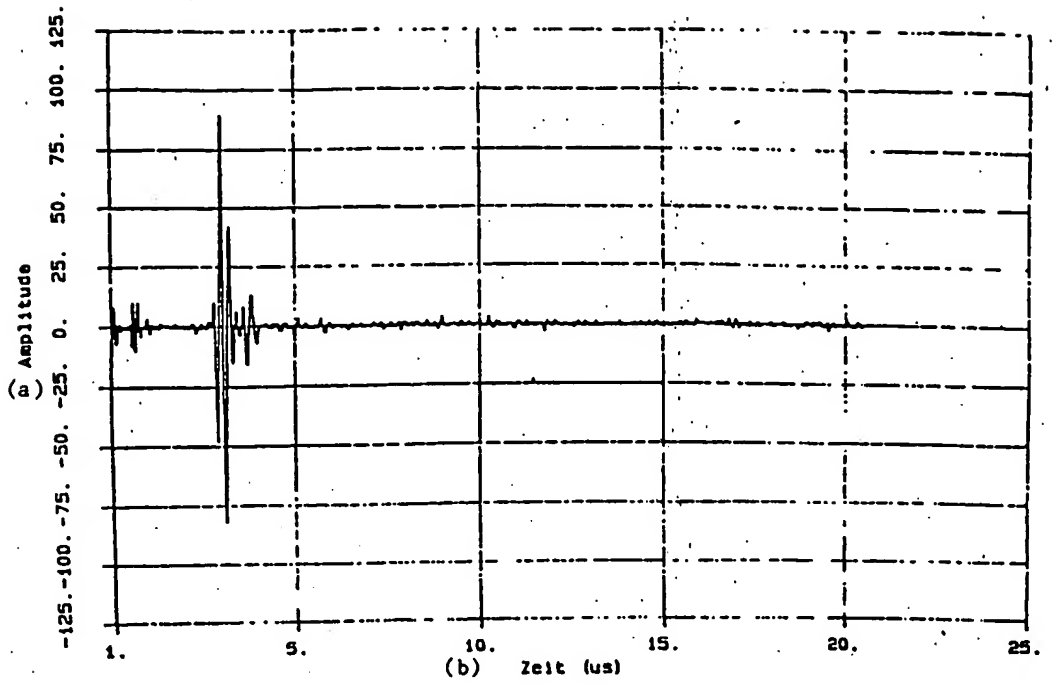


Figure 3. KEY: (a) amplitude; and (b) time, μs.

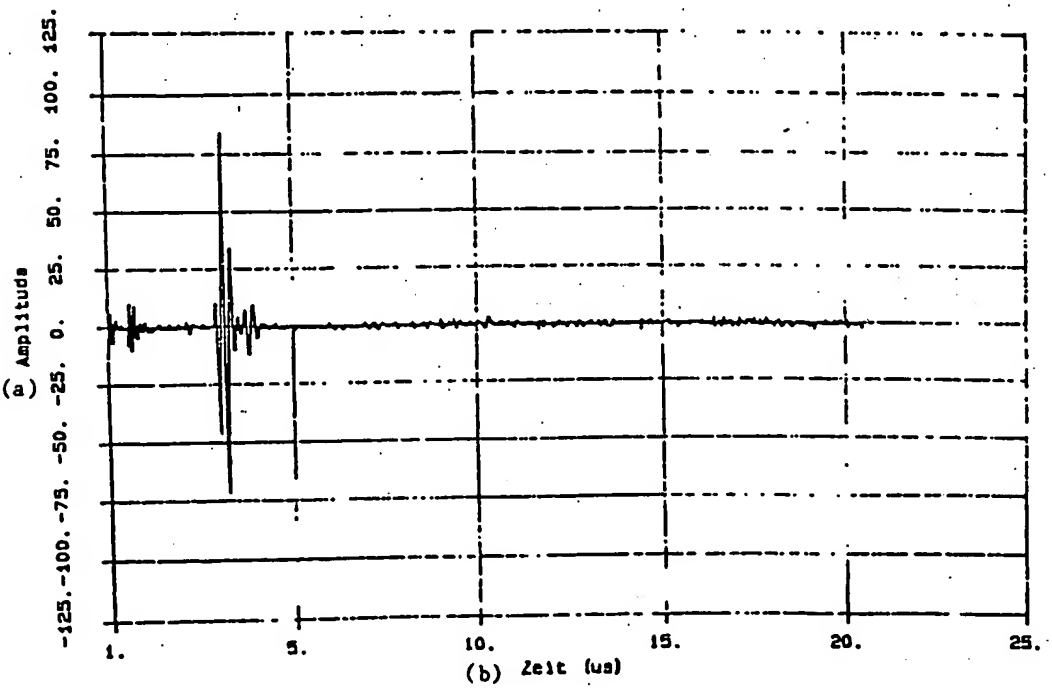


Figure 4. KEY: (a) amplitude; and (b) time, μs.

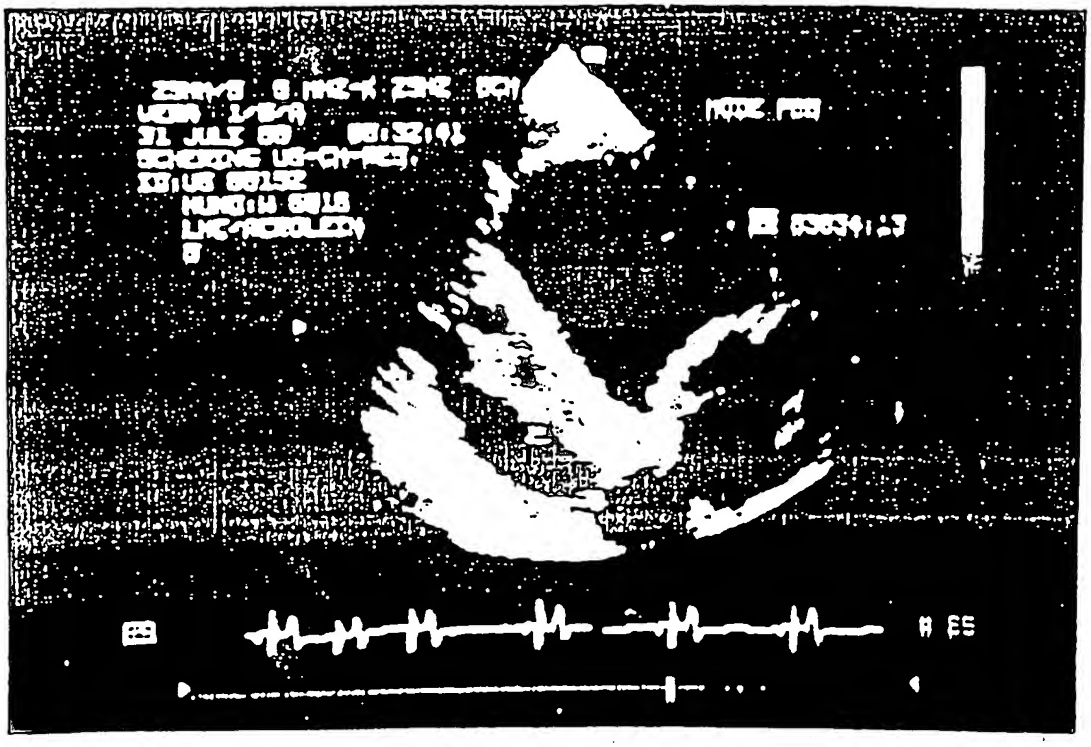


Figure 5. [*Legend is illegible -- Tr. Ed.*]

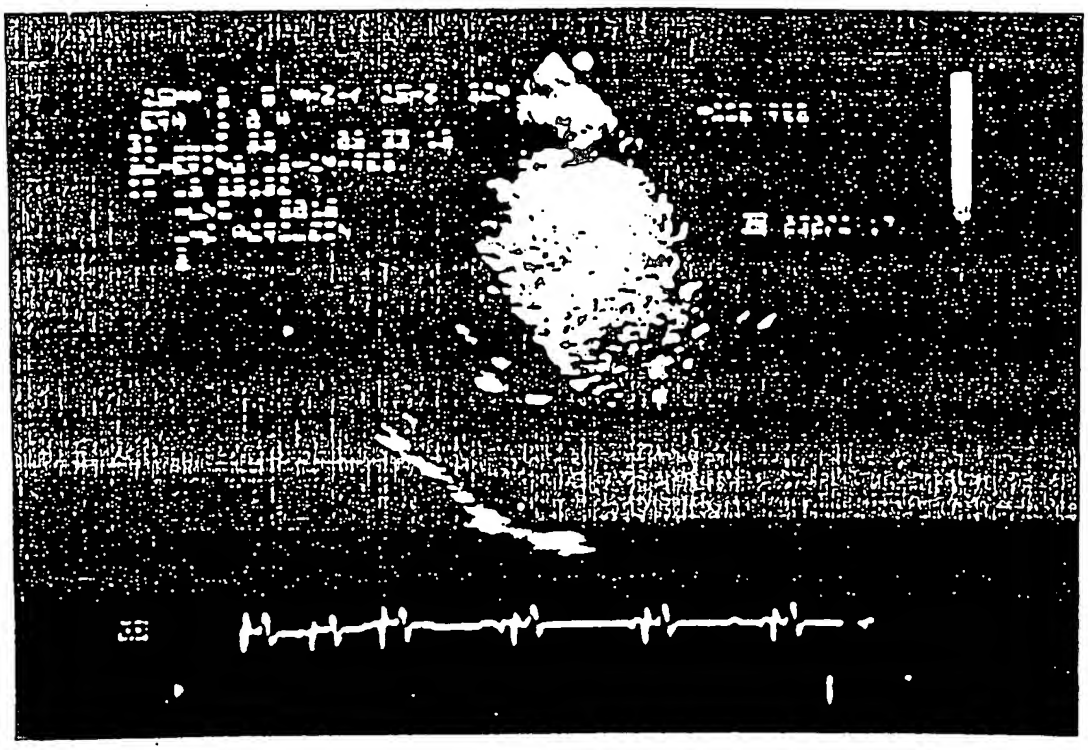


Figure 6. [*Legend is illegible -- Tr. Ed.*]